Discovery of Novel 2-Anilinopyrazolo[1,5-*a*]pyrimidine Derivatives as c-Src Kinase Inhibitors for the Treatment of Acute Ischemic Stroke

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We synthesized a series of novel 2-anilinopyrazolo[1,5-*a*]pyrimidine derivatives and evaluated their ability to inhibit c-Src kinase; 7-(2-amino-2-methylpropylamino)-5-cyclopropyl-2-(3,5-dimethoxyphenylamino)pyrazolo-[1,5-*a*]pyrimidine-3-carboxamide 7o and 7-(2-amino-2-methylpropylamino)-2-(3,5-dimethoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxamide 7f showed potent inhibitory activity. Compound 7f inhibited c-Src selectively and exhibited satisfactory central nervous system (CNS) penetration. Furthermore, 7f·HCl reduced the infarct volume *in vivo* in a rat middle cerebral artery (MCA) occlusion model when administrated intraperitoneally.

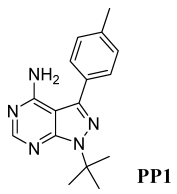
Key words c-Src kinase; pyrazolo[1,5-*a*]pyrimidine; acute ischemic stroke; central nervous system penetration

Stroke is a leading cause of death and disability worldwide, and the development of an effective therapeutic strategy for stroke has been a priority of neuroscientists for decades. Considerable effort has been devoted to developing neuroprotective agents to save neurons from the biochemical and metabolic consequences of ischemia brain injury. Although a variety of agents have demonstrated efficacy in reducing stroke injury in preclinical studies, only tissue plasminogen activator (t-PA) has been approved internationally as a drug for stroke patients. However, its narrow therapeutic window (3 h after stroke onset) and associated risks, including hemorrhage, have resulted in limited therapeutic use.[1)] Therefore, new and more useful agents are needed for treating ischemia/reperfusion injury.

Tyrosine kinase c-Src[2)] regulates the activity of the *N*-methyl-D-aspartate (NMDA) receptor, which induces a marked and prolonged Ca^{2+} influx into the neurons after cerebral ischemia[3,4)] and ultimately leads to neuronal damage. c-Src regulates the release of cytokine and superoxide production in neutrophils,[5,6)] and mediates the signaling activity in response to vascular endothelial growth factor (VEGF),[7)] which modulates vascular permeability and contributes to the formation of cerebral edema.[8,9)]

Mice lacking $pp60^{c\text{-}src}$ show a reduction in both infarct volume and VEGF-mediated vascular permeability after brain ischemia.[10)] Moreover, treatment of wild-type mice with PP1, a selective Src inhibitor, reduces infarct size and decreases edema, as shown in Fig. 1.[10—12)] Consequently, a selectively active inhibitor of tyrosine kinase c-Src would hold promise as an effective new medication for stroke.

Therefore, we started a program to discover a novel c-Src inhibitor as an agent for treating acute stroke. Screening of our compound library led to the discovery of compound **1** as a lead compound as an inhibitor of c-Src kinase. Compound **1** showed a high degree of selectivity for c-Src inhibition over other kinases (data not shown), and had a structurally novel pyrazolo[1,5-*a*]pyrimidine skeleton for a c-Src kinase inhibitor. Therefore, we sought to increase the inhibitory activity by varying this structure. In this paper, we describe the synthesis and structure–activity relationship (SAR) of 2-anilinopyrazolo[1,5-*a*]pyrimidine derivatives as c-Src kinase inhibitors and the neuroprotective effect of the resulting compounds in a rat model.



PP1

Fig. 1. Structure of the Src-Family Kinase Inhibitor PP1

Chemistry The general approach to the synthesis of 5,7-disubstituted 2-anilinopyrazolo[1,5-*a*]pyrimidine derivatives **7** and **8** is outlined in Chart 1. The intermediates **3** were prepared by substituting the corresponding anilines on [bis(methylthio)methylene]propanedinitrile **2**. Cyclization of **3** with hydrazine monohydrate produced 3-amino-5-anilinopyrazoles **4**. Treatment of **4** with β-keto ester derivatives under acidic conditions gave the 2-anilinopyrazolo[1,5-*a*]pyrimidine-7-one analogs **5**.[13)] The 7-chloro intermediates were obtained by converting carbonyl to chlorine with phosphorous oxychloride in *N*,*N*-dimethylaniline. Substitution of the 7-chlorine with alkylamines afforded 7-aminopyrazolo-[1,5-*a*]pyrimidines **6**. The amide derivatives **7** were prepared by hydrolyzing the corresponding nitriles **6** with basic hydrogen peroxide in dimethyl sulfoxide.[14)] Intermediates **7j**—**l** were deprotected with HCl/EtOAc to give the amide derivatives **8j**—**l**.

Result and Discussion

We evaluated the ability of the compounds to inhibit tyrosine phosphorylation by c-Src kinase. These data are shown in Tables 1 and 2. The pyrazolo[1,5-*a*]pyrimidine derivative **1** had potent c-Src inhibitory activity with an IC_{50} of 0.64 μM. First, we focused on the substituents (R^3) at the 7-position to improve the inhibitory activity (Table 1). Modification of the terminal primary amine to a secondary amine (**7a**) reduced the c-Src inhibitory activity compared to **1**, and the tertiary amine (**7b**) was less potent than **7a**. Replacing the amino

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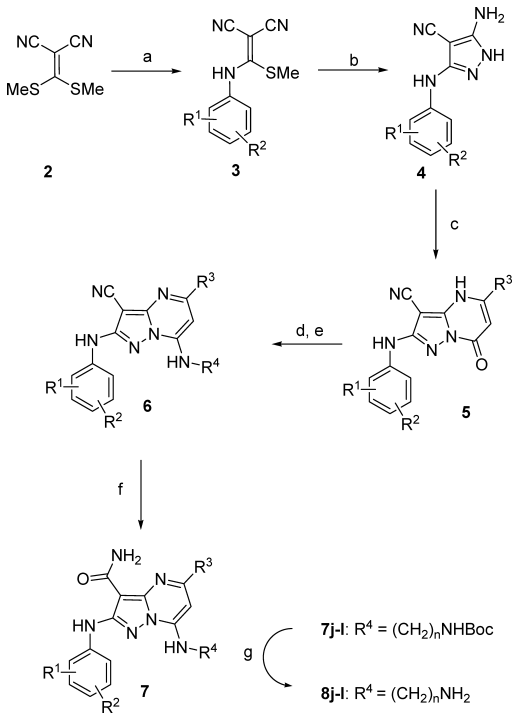
ethyl group (**1**) with a carbamoyl methyl (**7c**) or cyclopropyl methyl (**7d**, **e**) group resulted in a decrease in c-Src inhibitory activity. The chain length of the diamino moiety at the 7-position has a significant effect on the c-Src inhibitory activity. The inhibitory activity of the three-carbon chain derivative **8j** was inferior to that of the two-carbon chain derivative **1**. The introduction of a dimethyl group adjacent to the terminal nitrogen (**7f**) enhanced the inhibitory activity. In addition, the (*R*)-2′-aminopropyl analog **8k** showed superior activity to the (*S*)-2′-aminopropyl analog **8l**. These results suggest that both the terminal primary amine and the carbon chain length are important factors in the c-Src inhibitory activity, and that the 7-ethylenediamino group mimics the triphosphate group of adenosine triphosphate (ATP). These substituent effects were similar to reported effects for the Syk family of kinase inhibitors.[15,16]

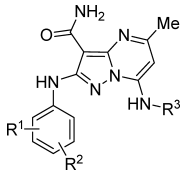
Next, we estimated the substituent effect of methoxy groups on the aniline phenyl group toward the inhibition of c-Src kinase. Previously, we reported that electron-donating substituents on the phenyl ring, especially the methoxy group, enhanced the c-Src inhibitory activity.[17] A single methoxy group at either the 3′- (**7g**) or 4′-position (**7h**) reduced the inhibitory activity against c-Src kinase compared to the dimethoxy derivative **7f**. Removing the methoxy group on the aniline phenyl ring (**7i**) resulted in activity similar to that of monomethoxy derivatives **7g** and **7h**. Conversely, 2′-



Reagents: (a) Substituted or unsubstituted aniline, MeOH, reflux; (b) hydrazine monohydrate, *N*,*N*-dimethylformamide (DMF), 100 °C; (c) $R^3(CO)CH_2CO_2Et$, AcOH, reflux; (d) $POCl_3$, *N*,*N*-dimethylaniline, 60 °C; (e) R^4NH_2, NEt_3, 1-methy-2-pyrrolidinone (NMP), r.t.; (f) NaOH, H_2O_2, 60 °C; (g) 4 M HCl, EtOAc, r.t.

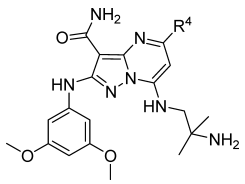
Chart 1

Table 1. Inhibitory Activities of Pyrazolo[1,5-*a*]pyrimidine Derivatives against c-Src Kinase



Compd.	R^1	R^2	R^3	c-Src IC_{50} (μM)
1	3′-MeO	5′-MeO	$-CH_2CH_2NH_2$	0.64
7a	3′-MeO	5′-MeO	$-CH_2CH_2NHMe$	2.73
7b	3′-MeO	5′-MeO	$-CH_2CH_2NMe_2$	27.80
7c	3′-MeO	5′-MeO	$-CH_2CONH_2$	33.70
7d	H	H	$-CH_2$cPr	2.48
7e	2′-MeO	H	$-CH_2$cPr	1000
7f	3′-MeO	5′-MeO	$-CH_2C(CH_3)_2NH_2$	0.18
7g	3′-MeO	H	$-CH_2C(CH_3)_2NH_2$	1.00
7h	4′-MeO	H	$-CH_2C(CH_3)_2NH_2$	1.22
7i	H	H	$-CH_2C(CH_3)_2NH_2$	1.71
8j	3′-MeO	5′-MeO	$-CH_2CH_2CH_2NH_2$	8.16
8k	3′-MeO	5′-MeO		0.41
8l	3′-MeO	5′-MeO		2.99

Table 2. Inhibitory Activities against c-Src Kinase and Selectivity Profiles of Pyrazolo[1,5-*a*]pyrimidine Derivatives



Compd.	R^4	IC_{50}(μM)						
		c-Src	Lck	ZAP	Syk	PKCβ	EGFR	KDR
7f	Me	0.175	0.962	44.6	2.62	1.07	5.95	1.00
7m	Ph	0.018	0.019	NT[a)]	NT[a)]	0.04	0.10	NT[a)]
7n	Et	0.010	0.288	27.1	3.89	5.97	1.80	NT[a)]
7o	cPr	0.004	0.010	124	3.72	1.70	0.01	NT[a)]
7p	iPr	0.021	0.099	46.6	12.9	1.84	0.42	NT[a)]
7q	nPr	0.028	0.350	91.6	11.9	0.82	0.14	NT[a)]
7r	CH_2COOH	0.651	1.970	NT[a)]	NT[a)]	8.65	6.99	NT[a)]

a) NT = not tested.

methoxy derivative **7e** showed no activity compared to the cyclopropylamino derivative **7d**. The complete disappearance of the inhibitory activity of compound **7e** may have been attributable to the steric clash between the 2′-methoxy group and the proximal amino acids of the binding site. These results indicate that a 3′,5′-dimethoxy group is a favorable substituent on the aniline phenyl ring in this series.

Thus, we investigated the influence of the substituent at the 5-position of the pyrazolo[1,5-*a*]pyrimidine core on the c-Src kinase inhibitory activity. The results are listed in Table 2. Aryl and heteroaryl groups are involved in a hydrophobic interaction with the hydrophobic pocket adjacent to the ATP-binding site, enhancing the tyrosine kinase inhibitory activity. For example, Chen *et al.* reported that replacing the aliphatic amines with aryl or heteroaryl amines at the 4-position of the 1,5-imidazoquinoxaline core improved the affinity with the hydrophobic pocket of Lck kinase.[18] Therefore, substituting the phenyl group at the 5-position (**7m**) increased the activity in comparison to **7f**. Interestingly, replacement with a sterically bulky alkyl group, even an ethyl group (**7n**), enhanced the activity more than that of the methyl analog **7f**. The introduction of bulkier substituents led to more active compounds **7p**, **q**. In this series, the 5-cyclopropyl derivative **7o** had the most potent activity (IC_{50}= 0.004 μM). Conversely, the additional introduction of a hydrophilic substituent (**7r**) led to loss of the inhibitory activity.

To gain insight into the structural basis of the inhibitory activity of our compounds, we conducted an X-ray analysis of the crystal structures of **7f** and **7o** co-crystallized with purified human Lck protein, a closely related member of the Src family, as shown in Fig. 2. The 3-carboxamide group and N–H of the 2-aminophenyl ring formed a pseudo-six-membered ring, and compounds **7f** and **7o** each interacted with several amino acid residues to enhance their binding affinity with the ATP binding site, as shown in Fig. 2a—c. For example, the N–H of the 3-carboxamide group formed a hydrogen bond with the carbonyl group of Glu 317 (Glu 341 in c-Src) and the carbonyl group of **7f** interacted with the N–H of Met 319 (Met 343 in c-Src). The N-4 of the pyrazolo[1,5-*a*]-pyrimidine formed a hydrogen bond with the hydroxyl group of Thr 316 (Thr 340 in c-Src). The terminal primary amino group of the 7-position was hydrogen bonded to the water bridging Asn 319.

Interestingly, five water molecules occupied the hydrophobic pocket (red balls; Fig. 2a), whereas the cyclopropyl group of **7o** extruded these water molecules to interact with the pocket (Fig. 2c). The cyclopropyl moiety was approximately 90° to the plane of the pyrazolo[1,5-*a*]pyrimidine nucleus. The results of the X-ray analysis explain the inhibitory activities obtained for the 5-substituted groups. Replacing the cluster of water molecules in the hydrophobic pocket with a hydrocarbon would stabilize the energy, as Fersht described previously.[19] The reasonable bulkiness and perpendicular conformation of the cyclopropyl group to the plane of the pyrazolo[1,5-*a*]pyrimidine nucleus seems to play an important role in binding through the stable hydrophobic interaction with the hydrophobic pocket.

Since selectivity against other kinase families is important for circumventing undesirable side effects, we evaluated the effects of compounds **7f** and **7m**—**r** on various kinases. These compounds showed excellent selectivity for inhibiting

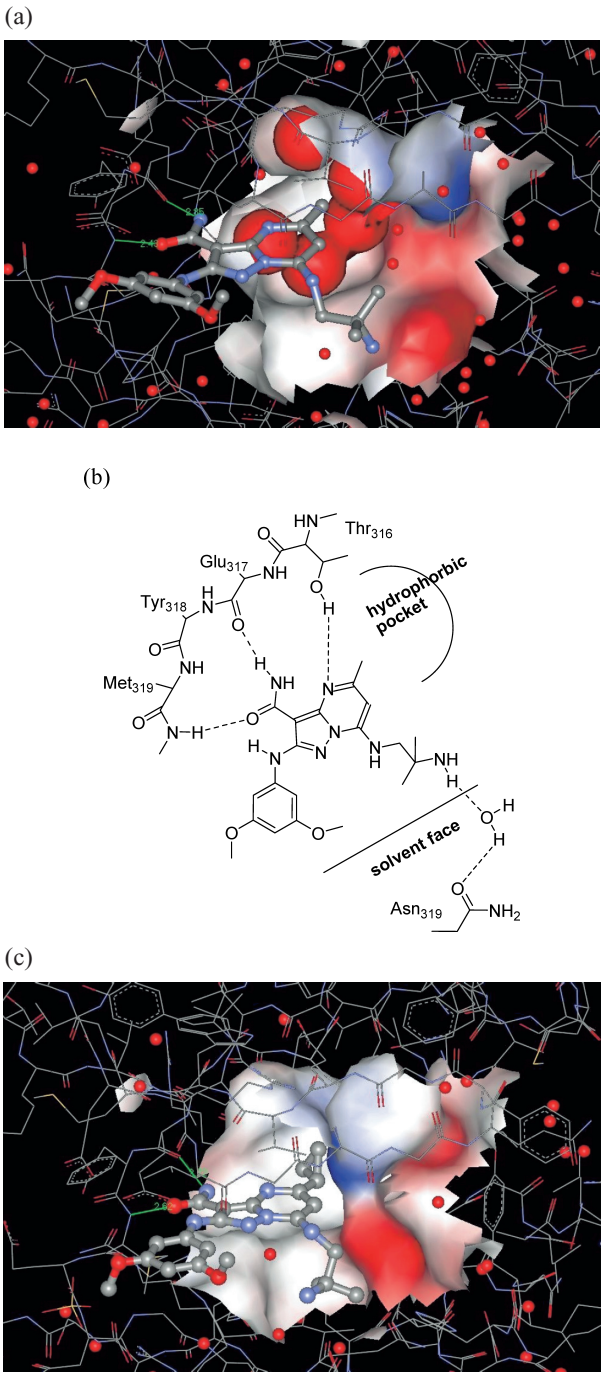


Fig. 2. Interactions of Compounds **7f** and **7o** with c-Src Kinases as Determined by X-Ray Crystallographic Studies

(a) The structure of the c-Src complex with **7f** is shown using a stick representation. Compound **7f** is shown as a ball and stick. The hydrophobic pocket is shown as a surface representation. Water molecules are represented as red balls. (b) Schematic representation of the binding mode of compound **7f** in c-Src kinase. (c) The structure of the c-Src complex with **7o** is shown using a stick representation. The hydrophobic pocket is shown as a surface representation.

c-Src kinase compared to the other kinases, such as Syk, Zap, PKCβ2, EGFR, and KDR kinases (see Table 2). Furthermore, these compounds exhibited greater selectivity for the inhibition of c-Src relative to Lck kinase, which is a family member with high sequence homology. The 5-cyclopropyl derivative **7o** had the best selectivity compared to Syk and Zap, but displayed significant activity against EGFR.

Based on the selective inhibition of c-Src kinase, com-

Table 3. Intracellular c-Src Kinase Inhibitory Activity of **7f·HCl** and Its Pharmacokinetic Parameters[a)]

Compd.	c-Src inhibition	CNS penetration (3 mg/kg/h, i.v. infusion)[b)]		
	ELISA IC_{50} (μM)	Plasma C_{3h} (ng/ml)	Brain C_{3h} (ng/g)	Ratio (brain/plasma)
7f	0.9	121.5±14.3	444.0±63.3	3.7±0.6

a) The results are the means±S.D. of three animals in each group. *b*) Compound **7f** was administered as a solution of EtOH : dimethyl isosorbide : propylene glycol 400 : 5% glucose (15 : 10 : 35 : 40).

Table 4. Pharmacokinetic Properties of Compound **7f·HCl** in Rats[a)]

Pharmacokinetic parameter	i.v.[b)]	i.p.[b)]
Dose (mg/kg)	1.0	3.0
$AUC_{0—inf.}$ (ng·h/ml)	292.0±50.4	—
$AUC_{0—4h}$ (ng·h/ml)	—	382.0±124.6
$C_{o\,max}$ (ng/ml)	190.3±41.1	—
C_{max} (ng/ml)	—	120.3±43.0
T_{max} (h)	—	1.5±0.9
$T_{1/2}$ (h)	2.8±1.0	—
MRT (h)	3.9±1.3	—
Cl (ml/min/kg)	58.3±9.2	—
Vss (l/kg)	13.4±4.4	—

a) The results are the mean±S.D. of three animals in each group. *b*) Compound **7f·HCl** was administered as a solution of 5% glucose.

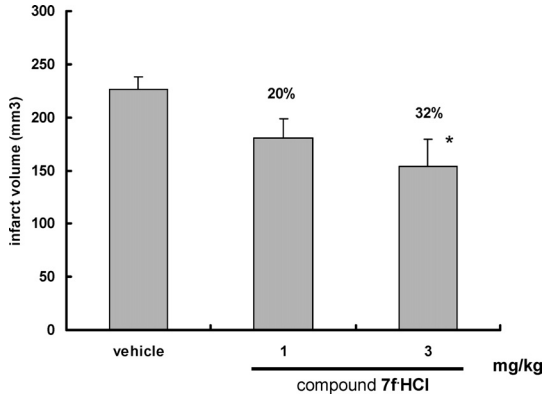


Fig. 3. Neuroprotective Effects of **7f·HCl** in a Middle Cerebral Artery (MCA) Thrombosis Model

$*p<0.05$ *vs.* vehicle (Dunnett's test; $n=6$—12).

pound **7f** was selected for an *in vivo* assay. Compound **7f** exhibited significant intracellular c-Src inhibition in the cellular assay, in an enzyme-linked immunosorbent assay (ELISA) using COS7 cells (Table 3).

To treat stroke effectively, it is important that compound **7f** penetrate the brain at pharmacologically active levels. We measured the concentration of **7f** in the brain and plasma 3 h after an intravenous infusion (Table 3). Compound **7f** had a significant brain/plasma ratio (3.7) and sufficient levels in the brain. In a pharmacokinetic study, **7f·HCl** was characterized as having a high volume of distribution (Vss) and a favorable half-life ($T_{1/2}$) and mean residence time (MRT) after intravenous administration (Table 4). In conjunction with the rat data 4 h after intraperitoneal administration, we concluded that **7f·HCl** had an appropriate pharmacokinetic profile for conducting *in vivo* efficacy studies.

We evaluated the *in vivo* activity of **7f·HCl** using the rat middle cerebral artery (MCA) occlusion model reported by Umemura *et al.*,[20] and assessed the neuroprotective effect using the infarct volume (see Fig. 3). Compound **7f·HCl** facilitated neuroprotection with a dose-dependent reduction in the infarct volume when administered intraperitoneally at doses of 1 or 3 mg/kg 15 min after MCA occlusion. In particular, **7f·HCl** at the highest dose (3 mg/kg) significantly reduced the infarct volume by 32% compared to the control.

Conclusion

We discovered a series of novel pyrazolo[1,5-*a*]pyrimidine derivatives possessing potent *in vitro* inhibitory activity against c-Src kinase. Various substituents at the 5- and 7-positions of the pyrazolo[1,5-*a*]pyrimidine played an important role in modulating the kinase inhibitory activity. Of these compounds, **7f·HCl** exhibited potent intracellular kinase inhibition and good CNS penetration. This compound also had a significant neuroprotective effect in rats, and could be a useful novel therapeutic agent for acute ischemic stroke.

Experimental

Biological Procedures. (a) Kinase Assays A coupled spectrophotometric assay was used wherein ADP generated by Src kinase was converted to ATP by pyruvate kinase (PK), with concomitant production of pyruvate from phosphoenolpyruvate (PEP). LDH reduces pyruvate to lactate by oxidizing NADH. NADH depletion was monitored at 340 nm using a microplate reader (Spectra Max 250, Molecular Device) at 30 °C for 20 min. Reactions were performed at 30 °C in 100 mM HEPES buffer (pH 7.6), containing 20 mM $MgCl_2$, and 10% glycerol, initiated by adding ATP. PK (100 μg/ml), LDH (50 μg/ml), PEP (2 mM), and NADH (140 μM) was also added. Kinase activity was measured by adding 100 μM Src optimal peptide substrate (peptide sequence: AEEEIYGEFEAKKKK, Sawady, Tokyo).

(b) Intracellular Kinase Inhibition Assay The kinase domain of human Src kinase (NM_005417, base# 790-1650) was cloned by PCR amplification, using the linker-containing primers 5′-AAACTTAAGCTTCATATGTCCAAGCCGCAGAC-3′ and 5′-CTGCAGATATCCCTAGAAGTAGTCCTCCAGGAA-3′. The gene for the Src kinase domain was integrated between the *Hin*dIII and *Eco*RV restriction sites within the multiple cloning site of the pcDNA3.1(+) expression vector. The vector was transfected into COS7 cells according to the calcium phosphate method.[21] Subsequently, 8.8 μg DNA was mixed with 220 μl 1 mM Tris–HCl, 0.1 mM EDTA buffer (pH 8.0), 250 μl 2×Hepes Buffered Saline, and 31 μl 2 M $CaCl_2$. The solution was incubated at room temperature for 15 min and then used to resuspend a pellet of 10^6 COS7 cells. The cell suspension was incubated at room temperature for 15 min; then, 4.5 ml Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum and antibiotics was added, and the solution was pre-warmed to 37 °C. Cells were seeded into 96-well multiwell plates with 2×10^4 cells/well/100 μl. A day after transfection, the culture media was replaced with media containing fixed concentrations of test compounds. On the third day, the cellular phosphotyrosine contents were determined using a commercially available phosphotyrosine ELISA kit (Upstate Biotechnology, NY, U.S.A. Cellular Phosphotyrosine ELISA Kit™, Catalog #17-182). Any basal phosphotyrosine content unrelated to c-Src activity was screened for using vector-transfected cells. Intracellular c-Src inhibition was expressed as the percentage of specific phosphotyrosine levels in drug-treated cells compared to the levels in control cells without drug. The toxicity of each chemical was determined with another replica plate using a colorimetric MTT assay kit (Chemicon International, CA, U.S.A. Cat. #CT02).

(c) Photochemically Induced Middle Cerebral Artery Occlusion in Rats Male Sprague-Dawley rats weighing 260—320 g were anesthetized with enflurane. The animal's body temperature was maintained at 37.5 °C with a heating pad. We performed middle cerebral artery (MCA) thrombosis as previously described.[20] A catheter was inserted into the femoral vein to administer the drug and rose bengal dye. The scalp and temporalis muscle were folded over. A subtemporal craniotomy was performed using a dental drill under an operating microscope to open a 3-mm-diameter oval bony window. The window was irradiated with green light (540 nm wavelength) using a xenon lamp (L4887, Hamamatsu Photonics, Hamamatsu, Japan), with both heat-absorbing and green filters. The irradiation was directed by a 3-mm-diameter optic fiber mounted on a micromanipulator. The head of the

optic fiber was placed on the window in the skull base at a distance of 2 mm above the vessel, providing an irradiation dose of 0.62 W/cm^2. Rose bengal (20 mg/kg) was injected intravenously. Photo-irradiation was continued for another 10 min. Compound **7f·HCl** was intraperitoneally administered at a dose of 1, and 3 mg/kg at 15 min after MCA occlusion. Twenty-four hours after surgery, the rats were sacrificed by administering an overdose of pentobarbital, and their brains were quickly removed. The cerebrum was separated from the other parts of the brain, and cut into six 2-mm-thick slices using a Brain Matrix (Muromachi Kikai, Japan). Each slice was incubated in 1% tetratrihydrochloride (TTC) solution at room temperature for 30 min and then photographed. The area of infarction was measured for each slice using a computerized image analysis system (Mac scope, Japan), and the ratio of infarction size was calculated by dividing the whole area by the region of cerebral ischemic damage.

(d) Analysis of c-Src Inhibitors in Plasma and Brain To inhibit c-Src kinase in the brain, we first determined the brain and plasma levels of the test compounds. Sprague-Dawley rats (300—350 g: $n=3$/study) were anesthetized with 25% urethane (1.1 g/kg, s.c.), and the femoral vein was cannulated to deliver the test compounds. Tracheal intubation was performed for artificial respiration. The animal's body temperature was maintained at 37.5 °C with a heating-pad. Compound **7f·HCl** was administered by intravenous infusion at 3 mg/kg/h. Three hours later, blood samples were taken from the aorta, and centrifuged to separate the plasma. Brain tissue was homogenized with a 3-fold volume of saline.

Plasma and brain tissue homogenate (50 μl) was mixed with 200 μl 100% acetonitrile, 100 μl 0.1% heptafluorobutyric acid, and 200 μl internal standard solution. After centrifugation at 3300 rpm for 20 min, the supernatant was removed and transferred to the column. Samples were quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The LC-MS/MS system consisted of an Alliance HPLC (Waters 2690) with a SYNERGI MAX-RP 80A column (4 μm, 50×4.6 mm, Phenomenex, Torrance, CA, U.S.A.), and a Sciex API 365 mass spectrometer (Perkin–Elmer Sciex, Toronto, Canada) equipped with a turbo ion spray source. The mobile phase consisted of 100% acetonitrile and 0.1% acetic acid in water (50 : 50 v/v). The column was maintained at 50 °C with a constant flow rate of 0.4 ml/min. The data was processed using Mass Chrom 1.1 software. The standard curves plotted for each test compound demonstrated good linearity (coefficient of determination >0.99). The limits of quantification of the compounds in plasma and brain were 50 ng/ml. All animal studies were approved by the Kissei Pharmaceutical Co., Ltd. Committee on Ethics of Animal Experimentation, and special care was taken to prevent animal suffering.

X-Ray Crystallographic Analysis of Human Lck Complex with 7f and 7o Human Lck was purified as described,[22] and concentrated to 3.0 mg/ml. Co-crystals were obtained at 277 K using the hanging-drop vapor-diffusion technique, equilibrated against a reservoir solution of 0.2 M $(NH_4)_2SO_4$, 0.1 M sodium cacodylate (pH 6.5), 30% (w/v) PEG8000. Before collecting the data, the crystals were quickly dipped in 20% glycerol containing a cryo-solution and plunged into liquid nitrogen. X-Ray diffraction data for the co-crystal of **7f** were collected at a wavelength of 1.0 Å with a BL32B2 station (SPring-8, Harima, Japan). The diffraction data of the co-crystal of **7o** were collected with an R-AXIS IV (Rigaku, Tokyo, Japan) diffractometer. The crystal complex with **7f** diffracted to 1.8 Å resolution and belongs to the $P2_12_12_1$ space group with $a=42.2$ Å, $b=74.0$ Å, and $c=91.66$ Å. The crystal complex with **7o** diffracted to 1.9 Å resolution and belongs to the $P2_12_12_1$ space group with $a=42.3$ Å, $b=73.8$ Å, and $c=93.0$ Å.

The diffraction data for the crystal complexes with **7f** and **7o** were integrated and scaled using the programs MOSFLM[23] and Scala[24] with an *R*sym(I) of 0.138 and a completeness of 98.8% to the highest resolution of 1.8 Å and with an *R*sym(I) of 0.124 and a completeness of 98.0% to the highest resolution of 1.9 Å, respectively.

The structures were refined using the program CNX (Accelrys, San Diego, CA, U.S.A.), starting with the existing refined Human Lck model structure (PDB code: 3lck), excluding the ligand. A rigid-body rotation–translation refinement was initially carried out to place the model structure more accurately in the new unit cell. Crystallographic refinement was continued by conjugate-gradient minimization and individual B factor refinement with CNX, and model/ligand building was performed with the program QUANTA (Accelrys).

The final structure complex with **7f** gave $R_{cryst}=0.199$ and $R_{free}=0.239$ at 1.8 Å resolution with good stereochemistry, a root mean square (rms) deviation from the ideal bond length of 0.0086 Å, and a root mean square (rms) deviation from the ideal bond angles of 1.23°.

The final structure complexed with **7o** gave $R_{cryst}=0.216$ and $R_{free}=0.252$ at 1.9 Å resolution with good stereochemistry, a root mean square (rms) deviation from the ideal bond length of 0.0087 Å, and a root mean square (rms) deviation from the ideal bond angle of 1.21°.

The geometry of both models was checked using the program PROCHECK[25]; no residues occurred in the disallowed regions of a Ramachandran plot.

Chemical Methods Melting points were taken on a Yanako MP-3S Micro melting point apparatus and are uncorrected. Infrared spectra were measured on a Nicolet 510 FT-IR spectrophotometer and are reported in reciprocal centimeters. Proton NMR spectra were recorded at 400 or 500 MHz with a Bruker AMX 400 or DRX 500 instrument, and chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane as the internal standard. The peak patterns are shown as the following abbreviations: br=broad, d=doublet, m=multiplet, s=singlet, t=triplet, q=quartet. The mass spectra (MS) were carried out with a Thermo Quest FINNIGAN AQA electrospray ionization mass spectrometer. Elemental analyses were performed on an Elementar Vario EL analyzer (C, H, N). The analytical results obtained were within ±0.4% of the theoretical values unless otherwise stated. Silica gel $60F_{254}$ precoated plates on glass from Merck KGaA or aminopropyl silica gel (APS) precoated NH plates from Fuji Silysia Chemical Ltd. were used for thin-layer chromatography (TLC). Flash column chromatography was performed on silica gel 60N (particle size 40—50 μm) from Kanto Chemical Co., Inc. or APS Daisogel IR-60 (particle size 25—40 μm) from Daiso Co., Ltd. All reagents and solvents were commercially available unless otherwise indicated.

2-Cyano-3-(3,5-dimethoxyphenylamino)-3-methylsulfanylacrylonitrile (3a) A mixture of 3,3-bis(methylsulfanyl)-2-cyanoacrylonitrile (**2**, 40.5 g, 0.24 mol) and 3,5-dimethoxyaniline (36.4 g, 0.24 mol) in MeOH (400 ml) was stirred for 15 h at reflux. After cooling at room temperature, collection of the resulting precipitates gave 53.4 g (82%) of **3a** as a white solid. ^{1}H-NMR ($CDCl_3$) δ: 2.33 (3H, s), 3.80 (6H, s), 6.40—6.45 (2H, m), 6.35—6.40 (1H, m).

2-Cyano-3-(3-methoxyphenylamino)-3-methylsulfanylacrylonitrile (3b) This title compound was prepared from **2** and 3-methoxyaniline according to the procedure described for preparation of **3a**, and obtained as an off-white solid (99% yield). ^{1}H-NMR ($CDCl_3$) δ: 2.33 (3H, s), 3.83 (3H, s), 6.79 (1H, t, $J=2.0$ Hz), 6.80—6.90 (2H, m), 7.33 (1H, t, $J=8.0$ Hz), 7.58 (1H, br s).

2-Cyano-3-(4-methoxyphenylamino)-3-methylsulfanylacrylonitrile (3c) This title compound was prepared from **2** and 4-methoxyaniline according to the procedure described for preparation of **3a**, and obtained as an off-white solid (93% yield). ^{1}H-NMR ($CDCl_3$) δ: 2.34 (3H, s), 3.84 (3H, s), 6.90—6.95 (2H, m), 7.15—7.20 (2H, m), 7.59 (1H, br s).

2-Cyano-3-methylsulfanyl-3-phenylaminoacrylonitrile (3d) This title compound was prepared from **2** and aniline according to the procedure described for preparation of **3a**, and obtained as an off-white solid (94% yield). ^{1}H-NMR ($CDCl_3$) δ: 2.29 (3H, s), 7.20—7.30 (2H, m), 7.30—7.40 (1H, m), 7.40—7.50 (2H, m), 7.82 (1H, br s).

2-Cyano-3-(2-methoxyphenylamino)-3-methylsulfanylacrylonitrile (3e) This title compound was prepared from **2** and 2-methoxyaniline according to the procedure described for preparation of **3a**, and obtained as an off-white solid (80% yield). ^{1}H-NMR (DMSO-d_6) δ: 2.60 (3H, s), 3.85 (3H, s), 6.95—7.05 (1H, m), 7.10—7.15 (1H, m), 7.20—7.25 (1H, m), 7.30—7.40 (1H, m), 10.14 (1H, br s).

5-Amino-3-(3,5-dimethoxyphenylamino)pyrazole-1*H*-4-carbonitrile (4a) A mixture of 2-cyano-3-(3,5-dimethoxyphenylamino)-3-methylsulfanylacrylonitrile (**3a**, 52.5 g, 0.19 mol) and hydrazine monohydrate (11.5 g, 0.23 mol) in *N*,*N*-dimethylformamide (110 ml) was stirred for 6 h at 100 °C. After cooling at room temperature, the mixture was diluted with water (500 ml) and stirred for 1 h. The resulting precipitate was collected by filtration and washed with water (500 ml) to give **4a** (45 g, 91%) as an off white solid. mp 249—250 °C (MeOH). ^{1}H-NMR (DMSO-d_6) δ: 3.67 (6H, s), 5.90—6.00 (1H, m), 6.25 (2H, br s), 6.70—6.75 (2H, m), 8.30 (1H, br s), 11.16 (1H, br s). IR (KBr) cm^{-1}: 3351, 2209, 1574, 1173. *Anal.* Calcd for $C_{12}H_{13}N_5O_2$: C, 55.59; H, 5.05; N, 27.01. Found: C, 55.20; H, 5.14; N, 27.05.

5-Amino-3-(3-methoxyphenylamino)pyrazole-1*H*-4-carbonitrile (4b) This title compound was prepared from **3b** according to the procedure described for preparation of **4a**, and obtained as an off-white solid (quant.). mp 182—183 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.69 (3H, s), 6.25 (2H, br s), 6.30—6.40 (1H, m), 7.00—7.10 (2H, m), 7.10—7.15 (1H, m), 8.30 (1H, s), 11.14 (1H, s). IR (KBr) cm^{-1}: 3385, 2198, 1617, 1498. *Anal.* Calcd for $C_{11}H_{11}N_5O$: C, 57.63; H, 4.84; N, 30.55. Found: C, 57.54; H, 4.88; N, 30.60.

5-Amino-3-(4-methoxyphenylamino)pyrazole-1*H*-4-carbonitrile (4c) This title compound was prepared from **3c** according to the procedure de-

scribed for preparation of **4a**, and obtained as an off-white solid (91%). mp 189—190 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.68 (3H, s), 6.20 (2H, br s), 6.77 (2H, d, J=9.1 Hz), 7.41 (2H, d, J=9.1 Hz), 8.05 (1H, s), 11.00 (1H, s). IR (KBr) cm^{-1}: 3289, 2212, 1511, 1247. *Anal.* Calcd for $C_{11}H_{11}N_5O$: C, 57.63; H, 4.84; N, 30.55. Found: C, 57.59; H, 4.87; N, 30.52.

5-Amino-3-phenylaminopyrazole-1*H*-4-carbonitrile (4d) This title compound was prepared from **3d** according the procedure to described for preparation of **4a**, and obtained as an off-white solid (87%). mp 204—205 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.24 (2H, br s), 6.70—6.80 (1H, m), 7.10—7.20 (2H, m) 7.35—7.55 (2H, m), 8.30 (1H, br s), 11,12 (1H, br s). IR (KBr) cm^{-1}: 3305, 2216, 1607, 1540, 1244. *Anal.* Calcd for $C_{10}H_9N_5$: C, 60.29; H, 4.55; N, 35.16. Found: C, 60.33; H, 4.59; N, 35.44.

5-Amino-3-(2-methoxyphenylamino)pyrazole-1*H*-4-carbonitrile (4e) This title compound was prepared from **3e** according to the procedure described for preparation of **4a**, and obtained as an off-white solid (87%). mp 165—166 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.87 (3H, s), 6.37 (2H, br s), 6.75 (1H, br s), 6.81 (1H, td, J=7.9, 1.6 Hz), 6.85 (1H, td, J=7.9, 1.6 Hz), 6.97 (1H, td, J=7.9, 1.6 Hz), 7.89 (1H, td, J=7.9, 1.6 Hz), 11.22 (1H, br s). IR (KBr) cm^{-1}: 3353, 2213, 1633, 1553, 1248. *Anal.* Calcd for $C_{11}H_{11}N_5O$: C, 57.63; H, 4.84; N, 30.55. Found: C, 57.60; H, 4.88; N, 30.74.

2-(3,5-Dimethoxyphenylamino)-5-methyl-7-oxo-4,7-dihydropyrazolo-[1,5-*a*]pyrimidine-3-carbonitrile (5a) A mixture of 5-amino-3-(3,5-dimethoxyphenylamino)pyrazole-1*H*-4-carbonitrile (**4a**, 8.2 g, 31.7 mmol) and ethyl acetoacetate (4.44 ml, 34.9 mmol) in acetic acid (80 ml) was stirred for 10 h at reflux. After cooling at room temperature, the mixture was diluted with water (80 ml) and stirred for 1 h. The resulting precipitates were collected by filtration and washed with water (200 ml) to give **5a** (9.4 g, 91%) as an off white solid. mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.29 (3H, s), 3.72 (6H, s), 5.79 (1H, br s), 6.10 (1H, t, J=2.2 Hz), 7.00 (2H, d, J=2.2 Hz), 9.15 (1H, br s), 13.04 (1H, br s). IR (KBr) cm^{-1}: 3355, 2215, 1598, 1560, 1155. *Anal.* Calcd for $C_{16}H_{15}N_5O_3\cdot 0.2H_2O$: C, 58.42; H, 4.72; N, 21.29. Found: C, 58.45; H, 4.61; N, 21.31.

2-(3-Methoxyphenylamino)-5-methyl-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-3-carbonitrile (5b) This title compound was prepared from **4b** according to the procedure described for preparation of **5a**, and obtained as an off-white solid (86%). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.29 (3H, s), 3.74 (3H, s), 5.79 (1H, s), 6.51 (1H, dd, J=8.2, 2.5 Hz), 7.18 (1H, t, J=8.2 Hz), 7.32 (1H, dd, J=8.2, 1.9 Hz), 7.38 (1H, t, J=2.4 Hz), 9.14 (1H, s), 13.00 (1H, s). IR (KBr) cm^{-1}: 3373, 3088, 2222, 1668, 1570, 1498. *Anal.* Calcd for $C_{15}H_{13}N_5O_2\cdot 0.25H_2O$: C, 60.09; H, 4.54; N, 23.36. Found: C, 59.84; H, 4.37; N, 23.22.

2-(4-Methoxyphenylamino)-5-methyl-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-3-carbonitrile (5c) This title compound was prepared from **4c** according to the procedure described for preparation of **5a**, and obtained as an off-white solid (97%). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.28 (3H, s), 3.72 (3H, s), 5.76 (1H, s), 6.88 (2H, d, J=8.8 Hz), 7.62 (2H, d, J=8.8 Hz), 8.92 (1H, s), 13.00 (1H, s). IR (KBr) cm^{-1}: 3343, 2215, 1684, 1639, 1594, 1559. *Anal.* Calcd for $C_{15}H_{13}N_5O_2\cdot 1.5H_2O$: C, 55.90; H, 5.00; N, 21.73. Found: C, 55.71; H, 4.91; N, 20.20.

5-Methyl-7-oxo-2-phenylamino-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-3-carbonitrile (5d) This title compound was prepared from **4d** according to the procedure described for preparation of **5a**, and obtained as an off-white solid (quant.). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.29 (3H, s), 5.79 (1H, s), 6.85—7.00 (1H, m), 7.25—7.35 (2H, m), 7.70—7.75 (2H, m), 9.16 (1H, s), 13.05 (1H, s). IR (KBr) cm^{-1}: 3415, 3072, 2215, 1668, 1624, 1596, 1559. *Anal.* Calcd for $C_{14}H_{11}N_5O$: C, 63.39; H, 4.18; N, 26.40. Found: C, 63.13; H, 4.04; N, 26.49.

2-(2-Methoxyphenylamino)-5-methyl-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-3-carbonitrile (5e) This title compound was prepared from **4e** according the procedure described for preparation of **5a**, and obtained as an off-white solid (98%). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.29 (3H, s), 3.89 (3H, s), 5.79 (1H, s), 6.90—7.10 (3H, m), 7.64 (1H, br s), 8.04 (1H, dd, J=7.6, 1.9 Hz), 13.06 (1H, br s). IR (KBr) cm^{-1}: 3415, 3091, 2214, 1676, 1635, 1594, 1559. *Anal.* Calcd for $C_{15}H_{13}N_5O_2\cdot 0.2H_2O$: C, 60.27; H, 4.52; N, 23.43. Found: C, 60.25; H, 4.38; N, 23.65.

5-Cyclopropyl-2-(3,5-dimethoxyphenylamino)-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-3-carbonitrile (5f) This title compound was prepared from **4a** and methyl 3-cyclopropyl-3-oxopropionate according the procedure to described for preparation of **5a**, and obtained as an off-white solid (73%). mp 186—187 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.92—1.14 (4H, m), 1.92—1.99 (1H, m), 3.72 (6H, s), 5.52 (1H, s), 6.10 (1H, t, J=2.2 Hz), 7.00 (2H, d, J=2.2 Hz), 9.11 (1H, s), 13.13 (1H, br s). IR (KBr) cm^{-1}: 2988, 2217, 1684,1635, 1529. *Anal.* Calcd for $C_{18}H_{17}N_5O_3$: C, 61.53; H, 4.88; N, 19.93. Found: C, 61.22; H, 4.85; N, 20.03.

2-(3,5-Dimethoxyphenylamino)-5-ethyl-7-oxo-4,7-dihydropyrazolo-[1,5-*a*]pyrimidine-3-carbonitrile (5g) This title compound was prepared from **4a** and ethyl 3-oxovalerate according to the procedure described for preparation of **5a**, and obtained as an off-white solid (88%). mp 270—272 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.22 (3H, t, J=7.6 Hz), 2.59 (2H, q, J=7.6 Hz), 3.72 (6H, s), 5.79 (1H, s), 6.10 (1H, t, J=2.2 Hz), 7.00 (2H, d, J=2.2 Hz), 9.12 (1H, s), 12.96 (1H, br s). IR (KBr) cm^{-1}: 3328, 2219, 1696, 1647, 1616, 1559. *Anal.* Calcd for $C_{17}H_{17}N_5O_3\cdot 1.25H_2O$: C, 56.42; H, 5.43; N, 19.35. Found: C, 56.65; H, 5.23; N, 19.64.

2-(3,5-Dimethoxyphenylamino)-7-oxo-5-phenyl-4,7-dihydropyrazolo-[1,5-*a*]pyrimidine-3-carbonitrile (5h) This title compound was prepared from **4a** and ethyl 3-oxo-3-phenylpropionate according to the procedure described for preparation of **5a**, and obtained as an off-white solid (76%). mp 274—275 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.73 (6H, s), 6.12 (1H, t, J=2.2 Hz), 6.20 (1H, s), 7.05 (2H, d, J=2.2 Hz), 7.50—7.65 (3H, m), 7.75—7.85 (2H, m), 9.15 (1H, s), 13.33 (1H, br s). IR (KBr) cm^{-1}: 3424, 2216, 1670, 1611, 1560. *Anal.* Calcd for $C_{21}H_{17}N_5O_3\cdot 0.2H_2O$: C, 64.51; H, 4.49; N, 17.91. Found: C, 64.56; H, 4.42; N, 18.26.

2-(3,5-Dimethoxyphenylamino)-5-isopropyl-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-3-carbonitrile (5i) This title compound was prepared from **4a** and methyl 4-methyl-3-oxopentanate according to the procedure described for preparation of **5a**, and obtained as an off-white solid (87%). mp 259—260 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.24 (6H, d, J=6.9 Hz), 2.85—2.95 (1H, m), 3.72 (6H, s), 5.79 (1H, s), 6.10 (1H, t, J=2.2 Hz), 7.00 (2H, d, J=2.2 Hz), 9.13 (1H, s), 12.88 (1H, br s). IR (KBr) cm^{-1}: 3380, 2219, 1692, 1639, 1593. *Anal.* Calcd for $C_{18}H_{19}N_5O_3\cdot 0.25H_2O$: C, 60.41; H, 5.49; N, 19.57. Found: C, 60.01; H, 5.14; N, 19.31.

2-(3,5-Dimethoxyphenylamino)-7-oxo-5-propyl-4,7-dihydropyrazolo-[1,5-*a*]pyrimidine-3-carbonitrile (5j) This title compound was prepared from **4a** and methyl butyrylacetate according to the procedure described for preparation of **5a**, and obtained as an off-white solid (92%). mp 261—262 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.94 (3H, t, J=7.3 Hz), 1.60—1.75 (2H, m), 2.54 (2H, t, J=7.6 Hz), 3.72 (6H, s), 5.80 (1H, s), 6.10 (1H, t, J=2.2 Hz), 7.00 (2H, d, J=2.2 Hz), 9.14 (1H, s), 12.96 (1H, br s). IR (KBr) cm^{-1}: 3317, 2223, 1696, 1614, 1560, 1474. *Anal.* Calcd for $C_{18}H_{19}N_5O_3\cdot H_2O$: C, 58.21; H, 5.70; N, 18.86. Found: C, 58.13; H, 5.62; N, 19.07.

Methyl [3-Cyano-2-(3,5-dimethoxyphenylamino)-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidin-5-yl]acetate (5k) This title compound was prepared from **4a** and dimethyl 3-oxoglutarate according to the procedure described for preparation of **5a**, and obtained as an off-white solid (78%). mp 280—281 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.70 (3H, s), 3.72 (6H, s), 3.80 (2H, s), 5.94 (1H, s), 6.11 (1H t, J=2.2 Hz), 7.00 (2H, d, J=2.2 Hz), 9.22 (1H, s), 13.25 (1H, br s). IR (KBr) cm^{-1}: 3393, 2215, 1630, 1617, 1565, 1483. *Anal.* Calcd for $C_{18}H_{17}N_5O_5$: C, 56.39; H, 4.47; N, 18.27. Found: C, 56.45; H, 4.59; N, 18.38.

2-(3,5-Dimethoxyphenylamino)-5-methyl-7-(2-methylaminoethylamino)pyrazolo[1,5-*a*]pyrimidine-3-carbonitrile (6a) (a) A mixture of 2-(3,5-dimethoxyphenylamino)-5-methyl-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-3-carbonitrile (**5a**, 3.61 g, 11.1 mmol) and in phosphorus oxychloride (21 ml, 0.22 mol) in *N*,*N*-dimethylaniline (21 ml, 0.17 mol) was stirred for 2 h at 60 °C. After cooling at room temperature, the mixture was poured into ice-water. The resulting precipitates were collected by filtration and washed with water (500 ml). After drying under vacuum, 3.7 g (96%) of 7-chloro-2-(3,5-dimethoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carbonitrile was obtained as a crude mixture. (b) To a suspension of 7-chloro-2-(3,5-dimethoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carbonitrile (222 mg, 0.65 mmol) in 1-methyl-2-pyrrolidinone (2.0 ml) was added 2-(methylamino)ethylamine (0.28 ml, 3.23 mmol), and the mixture was stirred for 24 h at room temperature. The resulting mixture was diluted with water (3.0 ml), and the obtained precipitates were collected by filtration and washed with water (3.0 ml) to give **6a** (226 mg, 92%) as an off white solid. mp 283—284 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.30 (3H, s), 2.40 (3H, s), 2.74 (2H, t, J=6.0 Hz), 3.43 (2H, t, J=6.0 Hz), 3.74 (6H, s), 6.08 (1H, t, J=2.2 Hz), 6.33 (1H, s), 6.98 (2H, d, J=2.2 Hz), 9.21 (1H, s). IR (KBr) cm^{-1}: 3310, 2210, 1593, 1584. *Anal.* Calcd for $C_{19}H_{23}N_7O_2$: C, 59.83; H, 6.08; N, 25.70. Found: C, 59.63; H, 6.01; N, 25.66.

2-(3,5-Dimethoxyphenylamino)-7-(2-dimethylaminoethylamino)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carbonitrile (6b) This title compound was prepared from **5a** according to the procedure described for preparation of **6a**, and obtained as an off-white solid (90%). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.21 (6H, s), 2.41 (3H, s), 2.50—2.60 (2H, m), 3.45 (2H, q, J=6.0 Hz), 3.74 (3H, s), 6.08 (1H, t, J=2.2 Hz), 6.33 (1H, s), 6.99 (2H, d, J=2.2 Hz), 7.24 (1H, t, J=5.0 Hz), 9.27 (1H, s). IR (KBr) cm^{-1}:

3315, 2208, 1622, 1593, 1487. *Anal.* Calcd for $C_{20}H_{25}N_7O_2 \cdot 0.25H_2O$: C, 60.06; H, 6.43; N, 24.51. Found: C, 60.20; H, 6.24; N, 24.31.

2-[3-Cyano-2-(3,5-dimethoxyphenylamino)-5-methylpyrazolo[1,5-*a*]-pyrimidin-7-ylamino]acetamide (6c) This title compound was prepared from **5a** according to the procedure described for preparation of **6a**, and obtained as an off-white solid (84%). mp 296—297 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.40 (3H, s), 3.74 (6H, s), 4.01 (2H, d, J=5.7 Hz), 6.09 (1H, t, J=2.2 Hz), 6.14 (1H, s), 7.01 (2H, d, J=2.2 Hz), 7.35 (1H, s), 7.65 (1H, s), 7.73 (1H, t, J=5.7 Hz), 9.25 (1H, s). IR (KBr) cm^{-1}: 3321, 3176, 2216, 1709, 1595, 1490. *Anal.* Calcd for $C_{18}H_{19}N_7O_3 \cdot 1.5H_2O$: C, 52.93; H, 5.43; N, 24.01. Found: C, 53.18; H, 5.35; N, 23.90.

7-Cyclopropylmethylamino-5-methyl-2-phenylaminopyrazolo[1,5-*a*]-pyrimidine-3-carbonitrile (6d) This title compound was prepared from **5d** according to the procedure described for preparation of **6a**, and obtained as an off-white solid (89%). mp 293—294 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.30—0.40 (2H, m), 0.45—0.55 (2H, m), 1.10—1.25 (1H, m), 2.40 (3H, s), 3.25—3.35 (2H, m), 6.37 (1H, s), 6.93 (1H, t, J=7.3 Hz), 7.25—7.35 (2H, m), 7.81 (2H, d, J=7.9 Hz), 7.91 (1H, t, J=6.4 Hz), 9.19 (1H, s). IR (KBr) cm^{-1}: 3324, 2206, 1609, 1594, 1569. *Anal.* Calcd for $C_{18}H_{18}N_6$: C, 67.90; H, 5.70; N, 26.40. Found: C, 67.69; H, 5.60; N, 26.59.

7-Cyclopropylmethylamino-2-(2-methoxyphenylamino)-5-methyl-pyrazolo[1,5-*a*]pyrimidine-3-carbonitrile (6e) This title compound was prepared from **5e** according to the procedure described for preparation of **6a**, and obtained as an off-white solid (92%). mp 198—199 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.30—0.40 (2H, m), 0.45—0.55 (2H, m), 1.15—1.25 (1H, m), 2.40 (3H, s), 3.25—3.35 (2H, m), 3.91 (3H, s), 6.39 (1H, s), 6.95—7.10 (3H, m), 7.60 (1H, br s), 8.00 (1H, t, J=6.3 Hz), 8.20—8.30 (1H, m). IR (KBr) cm^{-1}: 3410, 3331, 2202, 1607, 1590, 1560. *Anal.* Calcd for $C_{19}H_{20}N_6O \cdot 0.2H_2O$: C, 64.83; H, 5.84; N, 23.87. Found: C, 64.76; H, 5.68; N, 24.01.

7-(2-Amino-2-methylpropylamino)-2-(3,5-dimethoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carbonitrile (6f) This title compound was prepared from **5a** according to the procedure described for preparation of **6a**, and obtained as an off-white solid (89%). mp >300 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.09 (6H, s), 2.40 (3H, s), 3.20 (2H, s), 3.74 (6H, s), 6.06 (1H, t, J=2.2 Hz), 6.38 (1H, s), 7.04 (2H, d, J=2.2 Hz), 9.29 (1H, s). IR (KBr) cm^{-1}: 3321, 2205, 1623, 1594, 1487. *Anal.* Calcd for $C_{20}H_{25}N_7O_2$: C, 60.74; H, 6.37; N, 24.79. Found: C, 60.58; H, 6.33; N, 24.68.

7-(2-Amino-2-methylpropylamino)-2-(3-methoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carbonitrile (6g) This title compound was prepared from **5b** according to the procedure described for preparation of **6a**, and obtained as an off-white solid (86%). mp 269—270 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.10 (6H, s), 2.40 (3H, s), 3.22 (2H, s), 3.76 (3H, s), 6.40 (1H, s), 6.49 (1H, ddd, J=8.2, 2.2, 0.9 Hz), 7.15 (1H, t, J=8.2 Hz), 7.23 (1H, ddd, J=8.2, 2.2, 0.9 Hz), 7.52 (1H, t, J=2.2 Hz), 9.29 (1H, s). IR (KBr) cm^{-1}: 3330, 2200, 1591, 1536, 1480. *Anal.* Calcd for $C_{19}H_{23}N_7O$: C, 62.45; H, 6.34; N, 26.83. Found: C, 62.19; H, 6.29; N, 26.98.

7-(2-Amino-2-methylpropylamino)-2-(4-methoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carbonitrile (6h) This title compound was prepared from **5c** according to the procedure described for preparation of **6a**, and obtained as an off-white solid (90%). mp 225—226 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.09 (6H, s), 2.38 (3H, s), 3.23 (2H, s), 3.72 (3H, s), 6.39 (1H, s), 6.86 (2H, d, J =9.1 Hz), 7.65 (2H, d, J=9.1 Hz), 9.04 (1H, s). IR (KBr) cm^{-1}: 3333, 2204, 1595, 1568, 1512, 1235. *Anal.* Calcd for $C_{19}H_{23}N_7O$: C, 62.45; H, 6.34; N, 26.83. Found: C, 62.23; H, 6.29; N, 26.96.

7-(2-Amino-2-methylpropylamino)-5-methyl-2-phenylaminopyrazolo[1,5-*a*]pyrimidine-3-carbonitrile (6i) This title compound was prepared from **5d** according to the procedure described for preparation of **6a**, and obtained as an off-white solid (89%). mp 271—272 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.10 (6H, s), 2.39 (3H, s), 3.25 (2H, s), 6.42 (1H, s), 6.90—7.00 (1H, m), 7.20—7.35 (2H, m), 7.70—7.80 (2H, m), 9.24 (1H, s). IR (KBr) cm^{-1}: 3336, 2197, 1590, 1566, 1411. *Anal.* Calcd for $C_{18}H_{21}N_7$: C, 64.46; H, 6.31; N, 29.23. Found: C, 64.22; H, 6.23; N, 29.48.

***t*-Butyl 3-[3-Cyano-2-(3,5-dimethoxyphenylamino)-5-methylpyrazolo-[1,5-*a*]pyrimidin-7-ylamino]propylcarbamate (6j)** This title compound was prepared from **5a** according to the procedure described for preparation of **6a**, and obtained as an off-white solid (94%). mp 210—211 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.38 (9H, s), 1.60—1.80 (2H, m), 2.40 (3H, s), 2.95—3.05 (2H, m), 3.35—3.45 (2H, m), 3.73 (6H, s), 6.08 (1H, t, J=2.2 Hz), 6.31 (1H, br s), 6.92 (1H, t, J=5.7 Hz), 6.95 (2H, d, J=2.2 Hz), 7.79 (1H, t, J=6.3 Hz), 9.17 (1H, s). IR (KBr) cm^{-1}: 3307, 2211, 1690, 1617, 1540. *Anal.* Calcd for $C_{24}H_{31}N_7O_4$: C, 59.86; H, 6.49; N, 20.36. Found: C, 59.67; H, 6.49; N, 20.43.

***t*-Butyl (*R*)-2-[3-Cyano-2-(3,5-dimethoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidin-7-ylamino]-1-methylethylcarbamate (6k)** This title compound was prepared from **5a** according to the procedure described for preparation of **6a**, and obtained as an off-white solid (71%). mp 199—200 °C. $[\alpha]_D^{28}$ −15.4° (c=1.05, DMSO). ^{1}H-NMR (DMSO-d_6) δ: 1.09 (3H, d, J=6.0 Hz), 1.26 (9H, s), 2.40 (3H, s), 3.35—3.45 (2H, m), 3.74 (6H, s), 3.75—3.85 (1H, m), 6.08 (1H, t, J=2.2 Hz), 6.39 (1H, s), 6.87 (1H, d, J=7.9 Hz), 6.95 (2H, d, J=2.2 Hz), 7.67 (1H, t, J=5.7 Hz), 9.17 (1H, s). IR (KBr) cm^{-1}: 3362, 2212, 1684, 1616, 1594. *Anal.* Calcd for $C_{24}H_{31}N_7O_4$: C, 59.86; H, 6.49; N, 20.36. Found: C, 59.58; H, 6.44; N, 20.33.

***t*-Butyl (*S*)-2-[3-Cyano-2-(3,5-dimethoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidin-7-ylamino]-1-methylethylcarbamate (6l)** This title compound was prepared from **5a** according to the procedure described for preparation of **6a**, and obtained as an off-white solid (71%). mp 199—200 °C. $[\alpha]_D^{28}$ 14.7° (c=1.09, DMSO). ^{1}H-NMR (DMSO-d_6) δ: 1.09 (3H, d, J=6.0 Hz), 1.26 (9H, s), 2.40 (3H, s), 3.35—3.45 (2H, m), 3.74 (6H, s), 3.75—3.85 (1H, m), 6.08 (1H, t, J=2.2 Hz), 6.39 (1H, s), 6.87 (1H, d, J=7.9 Hz), 6.95 (2H, d, J=2.2 Hz), 7.67 (1H, t, J=5.7 Hz), 9.17 (1H, s). IR (KBr) cm^{-1}: 3345, 2212, 1683, 1617, 1568. *Anal.* Calcd for $C_{24}H_{31}N_7O_4 \cdot 0.25H_2O$: C, 59.31; H, 6.53; N, 20.17. Found: C, 59.30; H, 6.40; N, 20.26.

7-(2-Amino-2-methylpropylamino)-2-(3,5-dimethoxyphenylamino)-5-phenylpyrazolo[1,5-*a*]pyrimidine-3-carbonitrile (6m) This title compound was prepared from **5h** according to the procedure described for preparation of **6a**, and obtained as an off-white solid (81%). mp 166—167 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.13 (6H, s), 3.39 (2H, s), 3.75 (6H, s), 6.08 (1H, t, J=2.2 Hz), 7.03 (1H, s), 7.07 (2H, d, J=2.2 Hz), 7.50—7.60 (3H, m), 8.20—8.26 (2H, m), 9.42 (1H, s). IR (KBr) cm^{-1}: 3362, 2299, 1617, 1570. *Anal.* Calcd for $C_{25}H_{27}N_7O_2$: C, 65.63; H, 5.95; N, 21.43. Found: C, 65.32; H, 5.89; N, 21.34.

7-(2-Amino-2-methylpropylamino)-2-(3,5-dimethoxyphenylamino)-5-ethylpyrazolo[1,5-*a*]pyrimidine-3-carbonitrile (6n) This title compound was prepared from **5g** according to the procedure described for preparation of **6a**, and obtained as an off-white solid (75%). mp 250—251 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.10 (6H, s), 1.25 (3H, t, J=7.6 Hz), 2.66 (2H, q, J=7.6 Hz), 3.22 (2H, s), 3.74 (6H, s), 6.06 (1H, t, J=2.2 Hz), 6.38 (1H, s), 7.04 (2H, d, J=2.2 Hz), 9.32 (1H, s). IR (KBr) cm^{-1}: 3336, 2203, 1596, 1534, 1486. *Anal.* Calcd for $C_{21}H_{27}N_7O_2$: C, 61.60; H, 6.65; N, 23.94. Found: C, 61.31; H, 6.60; N, 23.55.

7-(2-Amino-2-methylpropylamino)-5-cyclopropyl-2-(3,5-dimethoxyphenylamino)pyrazolo[1,5-*a*]pyrimidine-3-carbonitrile (6o) This title compound was prepared from **5f** according to the procedure described for preparation of **6a**, and obtained as an off-white solid (68%). mp 246—247 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.95—1.05 (4H, m), 1.10 (6H, s), 2.00—2.10 (1H, m), 3.23 (2H, s), 3.73 (6H, s), 6.05 (1H, t, J=2.2 Hz), 6.43 (1H, s), 7.03 (2H, d, J=2.2 Hz), 9.28 (1H, s). IR (KBr) cm^{-1}: 3330, 2204, 1617, 1590, 1488. *Anal.* Calcd for $C_{22}H_{27}N_7O_2 \cdot 0.25H_2O$: C, 62.03; H, 6.51; N, 23.02. Found: C, 61.93; H, 6.33; N, 22.75.

7-(2-Amino-2-methylpropylamino)-2-(3,5-dimethoxyphenylamino)-5-isopropylpyrazolo[1,5-*a*]pyrimidine-3-carbonitrile (6p) This title compound was prepared from **5i** according to the procedure described for preparation of **6a**, and obtained as an off-white solid (62%). mp 129—130 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.10 (6H, s), 1.25 (6H, d, J=6.9 Hz), 2.85—3.00 (1H, m), 3.23 (2H, s), 3.74 (6H, s), 6.06 (1H, t, J=2.2 Hz), 6.37 (1H, s), 7.03 (2H, d, J=2.2 Hz), 9.78 (1H, s). IR (KBr) cm^{-1}: 3315, 2968, 2208, 1599, 1486. *Anal.* Calcd for $C_{22}H_{29}N_7O_2$: C, 62.39; H, 6.90; N, 23.15. Found: C, 62.08; H, 6.87; N, 22.97.

7-(2-Amino-2-methylpropylamino)-2-(3,5-dimethoxyphenylamino)-5-propylpyrazolo[1,5-*a*]pyrimidine-3-carbonitrile (6q) This title compound was prepared from **5j** according to the procedure described for preparation of **6a**, and obtained as an off-white solid (83%). mp 133—134 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.94 (3H, t, J=6.7 Hz), 1.10 (6H, s), 1.65—1.80 (2H, m), 2.55—2.65 (2H, m), 3.22 (2H, s), 3.74 (6H, s), 6.06 (1H, t, J=2.2 Hz), 6.38 (1H, s), 7.03 (2H, d, J=2.2 Hz), 9.29 (1H, s). IR (KBr) cm^{-1}: 3327, 2206, 1617, 1591, 1570. *Anal.* Calcd for $C_{22}H_{29}N_7O_2$: C, 62.39; H, 6.90; N, 23.15. Found: C, 62.05; H, 6.82; N, 22.78.

Methyl [7-(2-Amino-2-methylpropylamino)-3-cyano-2-(3,5-dimethoxyphenylamino)pyrazolo[1,5-*a*]pyrimidin-5-yl]acetate (6r) This title compound was prepared from **5k** according the procedure described for preparation of **6a**, and obtained as an off-white solid (68%). mp 217—218 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.11 (6H, s), 3.22 (2H, s), 3.66 (3H, s), 3.74 (6H, s), 3.77 (2H, s), 6.07 (1H, t, J=2.2 Hz), 6.49 (1H, s), 7.03 (2H, d, J=2.2 Hz), 9.35 (1H, s). IR (KBr) cm^{-1}: 3332, 2205, 1617, 1592, 1490. *Anal.* Calcd for $C_{22}H_{27}N_7O_4$: C, 58.27; H, 6.00; N, 21.62. Found: C, 58.33; H, 6.08; N, 21.94.

2-(3,5-Dimethoxyphenylamino)-5-methyl-7-(2-methylaminoethylamino)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (7a) To a solution of 2-(3,5-dimethoxyphenylamino)-5-methyl-7-(2-methylaminoethylamino)-pyrazolo[1,5-*a*]pyrimidine-3-carbonitrile (**6a**, 106 mg, 0.28 mmol) and 5 M aqueous NaOH (0.39 ml, 1.95 mmol) in DMSO (1 ml) and EtOH (1 ml) was added dropwise 30% aqueous hydrogen peroxide (0.22 ml, 1.95 mmol) at 60 °C, and the mixture was stirred for 3 h at same temperature. After cooling at room temperature, water (5.0 ml) was added to the mixture and the resulting precipitate was collected by filtration. The obtained solid was crystallized from MeOH and DMSO to give **7a** (100 mg, 90%) as an off white solid. mp 256—257 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.32 (3H, s), 2.43 (3H, s), 2.77 (2H, t, J=6.0 Hz), 3.46 (2H, t, J=6.0 Hz), 3.78 (6H, s), 6.09 (1H, t, J=2.2 Hz), 6.29 (1H, s), 6.90 (2H, d, J=2.2 Hz), 7.41 (1H, d, J=2.8 Hz), 7.61 (1H, d, J=2.8 Hz), 9.59 (1H, s). IR (KBr) cm^{-1}: 3374, 1647, 1604, 1566, 1458. *Anal.* Calcd for $C_{19}H_{25}N_7O_3 \cdot 0.25H_2O$: C, 56.49; H, 6.36; N, 24.27. Found: C, 56.32; H, 6.19; N, 24.20.

7-(2-Dimethylaminoethylamino)-2-(3,5-dimethoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxamide (7b) This title compound was prepared from **6b** according to the procedure described for preparation of **7a**, and obtained as an off-white solid (73%). mp 254—255 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.23 (6H, s), 2.44 (3H, s), 2.56 (2H, t, J=6.3 Hz), 3.45—3.55 (2H, m), 3.78 (6H, s), 6.09 (1H, t, J=2.2 Hz), 6.29 (1H, s), 6.90 (2H, d, J=2.2 Hz), 7.25 (1H, d, J=5.4 Hz), 7.42 (1H, d, J=2.8 Hz), 7.60 (1H, d, J=2.8 Hz), 9.60 (1H, s). IR (KBr) cm^{-1}: 3369, 1653, 1590, 1570, 1465. *Anal.* Calcd for $C_{20}H_{27}N_7O_3 \cdot 0.5H_2O$: C, 56.56; H, 6.68; N, 23.21. Found: C, 56.85; H, 6.40; N, 23.21.

7-Carbamoylmethylamino-2-(3,5-dimethoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxamide (7c) This title compound was prepared from **6c** according to the procedure described for preparation of **7a**, and obtained as an off-white solid (67%). mp 286—287 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.43 (3H, s), 3.78 (6H, s), 4.04 (2H, d, J=6.0 Hz), 6.09 (1H, s), 6.10 (1H, t, J=2.2 Hz), 6.91 (2H, d, J=2.2 Hz), 7.35 (1H, br s), 7.41 (1H, d, J=2.8 Hz), 7.61 (1H, d, J=2.8 Hz), 7.67 (1H, br s), 7.74 (1H, t, J=6.0 Hz), 9.61 (1H, s). IR (KBr) cm^{-1}: 3365, 1700, 1603, 1566, 1458. *Anal.* Calcd for $C_{18}H_{21}N_7O_4 \cdot 1.5H_2O$: C, 50.70; H, 5.67; N, 22.99. Found: C, 50.96; H, 5.54; N, 23.06.

7-Cyclopropylmethylamino-5-methyl-2-phenylaminopyrazolo[1,5-*a*]-pyrimidine-3-carboxamide (7d) This title compound was prepared from **6d** according to the procedure described for preparation of **7a**, and obtained as an off-white solid (85%). mp 246—247 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.30—0.40 (2H, m), 0.45—0.55 (2H, m), 1.15—1.30 (1H, m), 2.43 (3H, s), 3.35 (2H, t, J=6.6 Hz), 6.34 (1H, s), 6.94 (1H, t, J=7.3 Hz), 7.30—7.36 (2H, m), 7.38 (1H, d, J=2.8 Hz), 7.63 (1H, d, J=2.8 Hz), 7.75 (2H, d, J=7.6 Hz), 8.02 (1H, t, J=6.6 Hz), 9.63 (1H, s). IR (KBr) cm^{-1}: 3369, 3151, 1654, 1590, 1564. *Anal.* Calcd for $C_{18}H_{20}N_6O$: C, 64.27; H, 5.99; N, 24.98. Found: C, 64.02; H, 5.90; N, 25.02.

7-Cyclopropylmethylamino-2-(2-methoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxamide (7e) This title compound was prepared from **6e** according to the procedure described for preparation of **7a**, and obtained as an off-white solid (91%). mp 267—268 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.30—0.40 (2H, m), 0.45—0.55 (2H, m), 1.15—1.30 (1H, m), 2.43 (3H, s), 3.30—3.40 (2H, m), 3.90 (3H, s), 6.33 (1H, s), 6.85—6.95 (1H, m), 6.95—7.05 (2H, m), 7.22 (1H, br s), 7.61 (1H, br s), 7.95—8.05 (1H, m), 8.55—8.65 (1H, m), 9.94 (1H, s). IR (KBr) cm^{-1}: 3398, 3147, 1654, 1560, 1459. *Anal.* Calcd for $C_{19}H_{22}N_6O_2 \cdot 0.1H_2O$: C, 61.97; H, 6.08; N, 22.82. Found: C, 61.95; H, 5.96; N, 22.91.

7-(2-Amino-2-methylpropylamino)-2-(3,5-dimethoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxamide (7f) This title compound was prepared from **6f** according to the procedure described for preparation of **7a**, and obtained as an off-white solid (22%). mp 288—289 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.30 (6H, s), 2.45 (3H, s), 3.55 (2H, br s), 3.78 (6H, s), 6.06 (1H, t, J=2.2 Hz), 6.34 (1H, s), 6.95 (2H, d, J=2.2 Hz), 7.42 (1H, d, J=2.5 Hz), 7.59 (1H, d, J=2.5 Hz), 9.61 (1H, s). IR (KBr) cm^{-1}: 3355, 1654, 1593, 1472. *Anal.* Calcd for $C_{20}H_{27}N_7O_3 \cdot 0.25H_2O$: C, 57.47; H, 6.63; N, 23.64. Found: C, 57.51; H, 6.49; N, 23.49.

7-(2-Amino-2-methylpropylamino)-2-(3-methoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxamide (7g) This title compound was prepared from **6g** according to the procedure described for preparation of **7a**, and obtained as an off-white solid (91%). mp 243—244 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.11 (6H, s), 2.43 (3H, s), 3.24 (2H, s), 3.81 (3H, s), 6.35 (1H, s), 6.49 (1H, dd, J=8.2, 2.2 Hz), 7.04 (1H, dd, J=8.2, 2.2 Hz), 7.18 (1H, t, J=8.2 Hz), 7.38 (1H, br s), 7.55—7.65 (2H, m), 9.62 (1H, s). IR (KBr) cm^{-1}: 3351, 1649, 1590, 1566, 1547. *Anal.* Calcd for $C_{19}H_{25}N_7O_2$: C, 59.51; H, 6.57; N, 25.57. Found: C, 59.23; H, 6.50; N, 25.66.

7-(2-Amino-2-methylpropylamino)-2-(4-methoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxamide (7h) This title compound was prepared from **6h** according to the procedure described for preparation of **7a**, and obtained as an off-white solid (81%). mp 235—236 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.11 (6H, s), 2.41 (3H, s), 3.27 (2H, s), 3.73 (3H, s), 6.36 (1H, s), 6.89 (2H, d, J=8.8 Hz), 7.31 (1H, d, J=2.8 Hz), 7.58 (1H, d, J=2.8 Hz), 7.64 (2H, d, J=8.8 Hz), 9.40 (1H, br s). IR (KBr) cm^{-1}: 3346, 1653, 1590, 1562, 1233. *Anal.* Calcd for $C_{19}H_{25}N_7O_2$: C, 59.51; H, 6.57; N, 25.57. Found: C, 59.13; H, 6.46; N, 25.70.

7-(2-Amino-2-methylpropylamino)-5-methyl-2-phenylaminopyrazolo-[1,5-*a*]pyrimidine-3-carboxamide (7i) This title compound was prepared from **6i** according to the procedure described for preparation of **7a**, and obtained as an off-white solid (80%). mp 223—224 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.11 (6H, s), 2.42 (3H, s), 3.28 (2H, br s), 6.39 (1H, s), 6.93 (1H, t, J=7.3 Hz), 7.25—7.35 (2H, m), 7.37 (1H, d, J=2.8 Hz), 7.60 (1H, d, J=2.8 Hz), 7.71 (2H, d, J=7.9 Hz), 9.62 (1H, s). IR (KBr) cm^{-1}: 3355, 1648, 1590, 1564. *Anal.* Calcd for $C_{18}H_{23}N_7O \cdot 0.25H_2O$: C, 60.40; H, 6.62; N, 27.39. Found: C, 60.33; H, 6.41; N, 27.65.

***t*-Butyl 3-[3-Carbamoyl-2-(3,5-dimethoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidin-7-ylamino]propylcarbamate (7j)** This title compound was prepared from **6j** according to the procedure described for preparation of **7a**, and obtained as an off-white solid (94%). mp 231—232 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.39 (9H, s), 1.65—1.80 (2H, m), 2.43 (3H, s), 2.95—3.10 (2H, m), 3.35—3.50 (2H, m), 3.77 (6H, s), 6.10 (1H, t, J=2.2 Hz), 6.27 (1H, s), 6.87 (2H, d, J=2.2 Hz), 6.95 (1H, t, J=5.7 Hz), 7.39 (1H, d, J=3.0 Hz), 7.63 (1H, d, J=3.0 Hz), 7.70 (1H, t, J=6.4 Hz), 9.58 (1H, s). IR (KBr) cm^{-1}: 3364, 1602, 1569, 1457, 1167. *Anal.* Calcd for $C_{24}H_{33}N_7O_5$: C, 57.70; H, 6.66; N, 19.63. Found: C, 57.58; H, 6.60; N, 19.61.

***t*-Butyl (*R*)-2-[3-Carbamoyl-2-(3,5-dimethoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidin-7-ylamino]-1-methylethylcarbamate (7k)** This title compound was prepared from **6k** according to the procedure described for preparation of **7a**, and obtained as an off-white solid (89%). mp 227—228 °C. $[\alpha]_D^{28}$ −10.3° (c=1.01, DMSO). ^{1}H-NMR (DMSO-d_6) δ: 1.11 (3H, d, J=6.9 Hz), 1.28 (9H, s), 2.43 (3H, s), 3.30—3.50 (2H, m), 3.75—3.90 (1H, m), 3.77 (6H, s), 6.10 (1H, t, J=2.2 Hz), 6.35 (1H, s), 6.88 (2H, d, J=2.2 Hz), 6.90—6.95 (1H, m), 7.39 (1H, d, J=2.5 Hz), 7.55—7.70 (2H, m), 9.57(1H, s). IR (KBr) cm^{-1}: 3353, 1696, 1590, 1458. *Anal.* Calcd for $C_{24}H_{33}N_7O_5 \cdot 0.5H_2O$: C, 56.68; H, 6.74; N, 19.28. Found: C, 56.29; H, 6.61; N, 19.32.

***t*-Butyl (*S*)-2-[3-Carbamoyl-2-(3,5-dimethoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidin-7-ylamino]-1-methylethylcarbamate (7l)** This title compound was prepared from **6l** according to the procedure described for preparation of **7a**, and obtained as an off-white solid (81%). mp 226—227 °C. $[\alpha]_D^{28}$ 13.7° (c=1.02, DMSO). ^{1}H-NMR (DMSO-d_6) δ: 1.11 (3H, d, J=6.9 Hz), 1.28 (9H, s), 2.43 (3H, s), 3.30—3.50 (2H, m), 3.75—3.90 (1H, m), 3.77 (6H, s), 6.10 (1H, t, J=2.2 Hz), 6.35 (1H, s), 6.88 (2H, d, J=2.2 Hz), 6.90—6.95 (1H, m), 7.39 (1H, d, J=2.5 Hz), 7.55—7.70 (2H, m), 9.57 (1H, s). IR (KBr) cm^{-1}: 3353, 1696, 1590, 1458. *Anal.* Calcd for $C_{24}H_{33}N_7O_5 \cdot 0.5H_2O$: C, 56.68; H, 6.74; N, 19.28. Found: C, 56.72; H, 6.60; N, 19.48.

7-(2-Amino-2-methylpropylamino)-2-(3,5-dimethoxyphenylamino)-5-phenylpyrazolo[1,5-*a*]pyrimidine-3-carboxamide (7m) This title compound was prepared from **6m** according to the procedure described for preparation of **7a**, and obtained as an off-white solid (80%). mp 288—289 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.15 (6H, s), 3.41 (2H, s), 3.79 (6H, s), 6.08 (1H, t, J=2.2 Hz), 6.95—7.00 (3H, m), 7.45—7.60 (4H, m), 7.69 (1H, d, J=2.8 Hz), 8.20—8.25 (2H, m), 9.69 (1H, s). IR (KBr) cm^{-1}: 3424, 1648, 1592, 1571, 1457. *Anal.* Calcd for $C_{25}H_{29}N_7O_3 \cdot 0.5H_2O$: C, 61.97; H, 6.24; N, 20.23. Found: C, 61.79; H, 6.05; N, 20.22.

7-(2-Amino-2-methylpropylamino)-2-(3,5-dimethoxyphenylamino)-5-ethylpyrazolo[1,5-*a*]pyrimidine-3-carboxamide (7n) This title compound was prepared from **6n** according to the procedure described for preparation of **7a**, and obtained as an off-white solid (76%). mp 245—246 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.12 (6H, s), 1.27 (3H, t, J=7.6 Hz), 2.70 (2H, q, J=7.6 Hz), 3.24 (2H, s), 3.77 (6H, s), 6.06 (1H, t, J=2.2 Hz), 6.34 (1H, s), 6.95 (2H, d, J=2.2 Hz), 7.42 (1H, d, J=2.8 Hz), 7.62 (1H, d, J=2.8 Hz), 9.59 (1H, s). IR (KBr) cm^{-1}: 3349, 1654, 1593, 1564, 1464. *Anal.* Calcd for $C_{21}H_{29}N_7O_3 \cdot 0.4H_2O$: C, 58.02; H, 6.91; N, 22.55. Found: C, 58.23; H, 6.87; N, 22.18.

7-(2-Amino-2-methylpropylamino)-5-cyclopropyl-2-(3,5-dimethoxyphenylamino)pyrazolo[1,5-*a*]pyrimidine-3-carboxamide (7o) This title compound was prepared from **6o** according to the procedure described for preparation of **7a**, and obtained as an off-white solid (72%). mp 246—247 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.00—1.05 (4H, m), 1.12 (6H, s), 2.00—

2.10 (1H, m), 3.24 (2H, m), 3.77 (6H, s), 6.06 (1H, t, J=2.2 Hz), 6.40 (1H, s), 6.94 (2H, d, J=2.2 Hz), 7.36 (1H, d, J=2.5 Hz), 7.43 (1H, d, J=2.5 Hz), 9.58 (1H, s). IR (KBr) cm^{-1}: 3403, 1654, 1589, 1472. *Anal.* Calcd for $C_{22}H_{29}N_7O_3 \cdot 0.2H_2O$: C, 59.63; H, 6.69; N, 22.13. Found: C, 59.58; H, 6.55; N, 22.14.

7-(2-Amino-2-methylpropylamino)-2-(3,5-dimethoxyphenylamino)-5-isopropylpyrazolo[1,5-*a*]pyrimidine-3-carboxamide (7p) This title compound was prepared from **6p** according to the procedure described for preparation of **7a**, and obtained as an off-white solid (85%). mp 235—236 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.12 (6H, s), 1.27 (6H, d, J=6.9 Hz), 2.90—3.00 (1H, m), 3.26 (2H, s), 3.77 (6H, s), 6.07 (1H, t, J=2.2 Hz), 6.33 (1H, s), 6.96 (2H, d, J=2.2 Hz), 7.42 (1H, d, J=2.8 Hz), 7.63 (1H, d, J=2.8 Hz), 9.58 (1H, s). IR (KBr) cm^{-1}: 3323, 1654, 1590, 1563, 1459. *Anal.* Calcd for $C_{22}H_{31}N_7O_3 \cdot 0.2H_2O$: C, 59.36; H, 7.11; N, 22.03. Found: C, 59.25; H, 6.90; N, 21.78.

7-(2-Amino-2-methylpropylamino)-2-(3,5-dimethoxyphenylamino)-5-propylpyrazolo[1,5-*a*]pyrimidine-3-carboxamide (7q) This title compound was prepared from **6q** according to the procedure described for preparation of **7a**, and obtained as an off-white solid (42%). mp 252—253 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.95 (3H, t, J=7.3 Hz), 1.11 (6H, s), 1.70—1.80 (2H, m), 2.65 (2H, t, J=7.6 Hz), 3.24 (2H, s), 3.77 (6H, s), 6.06 (1H, t, J=2.2 Hz), 6.33 (1H, s), 6.95 (2H, d, J=2.2 Hz), 7.41 (1H, d, J=2.8 Hz), 7.61 (1H, d, J=2.8 Hz), 9.59 (1H, s). IR (KBr) cm^{-1}: 3371, 3153, 1653, 1593, 1458. *Anal.* Calcd for $C_{22}H_{31}N_7O_3$: C, 59.85; H, 7.08; N, 22.21. Found: C, 59.59; H, 7.01; N, 22.14.

[7-(2-Amino-2-methylpropylamino)-3-carbamoyl-2-(3,5-dimethoxyphenylamino)pyrazolo[1,5-*a*]pyrimidin-5-yl]acetic acid (7r) This title compound was prepared from **6r** according to the procedure described for preparation of **7a**, and obtained as an off-white solid (74%). mp 282—283 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.20 (6H, s), 3.48 (2H, s), 3.57 (2H, s), 3.77 (6H, s), 6.08 (1H, t, J=2.2 Hz), 6.57 (1H, s), 6.95 (2H, d, J=2.2 Hz), 7.35—7.40 (1H, m), 7.55—7.65 (1H, m), 9.59 (1H, s). IR (KBr) cm^{-1}: 3384, 1653, 1576, 1364. *Anal.* Calcd for $C_{21}H_{27}N_7O_5 \cdot 4.25H_2O$: C, 47.23; H, 6.70; N, 18.36. Found: C, 47.12; H, 6.63; N, 18.50.

7-(2-Amino-2-methylpropylamino)-2-(3,5-dimethoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxamide hydrochloride (7f·HCl) To a solution of 7-(2-amino-2-methylpropylamino)-2-(3,5-dimethoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxamide (**7f**, 240 mg, 0.58 mmol) in MeOH (3 ml) was added 4 M HCl/EtOAc (0.22 ml, 0.87 mmol). The mixture was stirred for 1 h at room temperature, and evaporated *in vacuo*. The residue was crystallized from MeOH to give **7f·HCl** (196 mg, 75%) as a white solid. mp 291—292 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.30 (6H, s), 2.45 (3H, s), 3.57 (2H, s), 3.78 (6H, s), 6.09 (1H, t, J=2.2 Hz), 6.56 (1H, s), 6.93 (2H, d, J=2.2 Hz), 7.10—7.80 (3H, m), 7.42 (1H, d, J=2.5 Hz), 7.61 (1H, d, J=2.5 Hz), 9.61 (1H, s). IR (KBr) cm^{-1}: 3383, 2963, 1643, 1594, 1574, 1150. *Anal.* Calcd for $C_{20}H_{27}N_7O_3 \cdot HCl$: C, 53.39; H, 6.27; N, 21.79. Found: C, 53.62; H, 6.36; N, 21.92.

7-(3-Aminopropylamino)-2-(3,5-dimethoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxamide (8j) To a solution of *t*-butyl 3-[3-carbamoyl-2-(3,5-dimethoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidin-7-ylamino]propylcarbamate (**7j**, 95 mg, 0.19 mmol) in MeOH (2 ml) was added 4 M HCl/EtOAc (210 μl, 0.95 mmol). The mixture was stirred for 2 h at room temperature, and evaporated *in vacuo*. The residue was triturated with the saturated aqueous sodium hydrogen carbonate solution, and the resulting precipitate was collected by filtration to give **8j** (75 mg, 90%) as a white solid. mp 125—126 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.65—1.80 (2H, m), 2.43 (3H, s), 2.75 (2H, t, J=6.3 Hz), 3.49 (2H, t, J=6.3 Hz), 3.76 (6H, s), 6.10 (1H, t, J=2.2 Hz), 6.23 (1H, s), 6.90 (2H, d, J=2.2 Hz), 7.36 (1H, d, J=2.8 Hz), 7.61 (1H, d, J=2.8 Hz), 9.54 (1H, s). IR (KBr) cm^{-1}: 3158, 1680, 1614, 1464. *Anal.* Calcd for $C_{19}H_{25}N_7O_3 \cdot 0.25H_2O$: C, 56.49; H, 6.36; N, 24.27. Found: C, 56.41; H, 6.23; N, 24.42.

7-((*R*)-2-Aminopropylamino)-2-(3,5-dimethoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxamide (8k) This title compound was prepared from **7k** according to the procedure described for preparation of **8j**, and obtained as an off-white solid (61%). mp 269—270 °C. $[\alpha]_D^{28}$ −8.7° (c=1.06, DMSO). ^{1}H-NMR (DMSO-d_6) δ: 1.07 (3H, d, J=6.0 Hz), 2.43 (3H, s), 3.05—3.20 (2H, m), 3.30—3.40 (1H, m), 3.77 (6H, s), 6.08 (1H, t, J=2.2 Hz), 6.31 (1H, s), 6.91 (2H, d, J=2.2 Hz), 7.39 (1H, d, J=2.6 Hz), 7.61 (1H, d, J=2.6 Hz), 9.59 (1H, s). IR (KBr) cm^{-1}: 3189, 1671, 1602, 1465, 1205. *Anal.* Calcd for $C_{19}H_{25}N_7O_3 \cdot 0.25H_2O$: C, 56.49; H, 6.36; N, 24.27. Found: C, 56.46; H, 6.26; N, 23.92.

7-((*S*)-2-Aminopropylamino)-2-(3,5-dimethoxyphenylamino)-5-methylpyrazolo[1,5-*a*]pyrimidine-3-carboxamide (8l) This title compound was prepared from **7l** according to the procedure described for preparation of **8j**, and obtained as an off-white solid (79%). mp 272—273 °C. $[\alpha]_D^{28}$ 8.4° (c=1.02, DMSO). ^{1}H-NMR (DMSO-d_6) δ: 1.07 (3H, d, J=6.0 Hz), 2.43 (3H, s), 3.05—3.20 (2H, m), 3.30—3.40 (1H, m), 3.77 (6H, s), 6.08 (1H, t, J=2.2 Hz), 6.31 (1H, s), 6.91 (2H, d, J=2.2 Hz), 7.39 (1H, d, J=2.6 Hz), 7.61 (1H, d, J=2.6 Hz), 9.59 (1H, s). IR (KBr) cm^{-1}: 3178, 1670, 1599, 1458, 1205. *Anal.* Calcd for $C_{19}H_{25}N_7O_3 \cdot 0.25H_2O$: C, 56.49; H, 6.36; N, 24.27. Found: C, 56.16; H, 6.22; N, 23.98.

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